Культивирование и идентификация прокариотических клеток

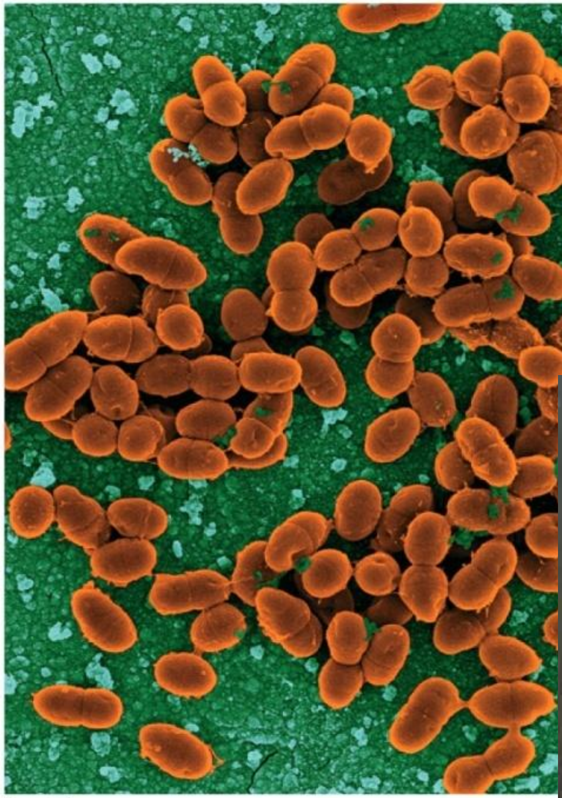

Figure 1-1a
Molecular Cell Biology, Sixth Edition
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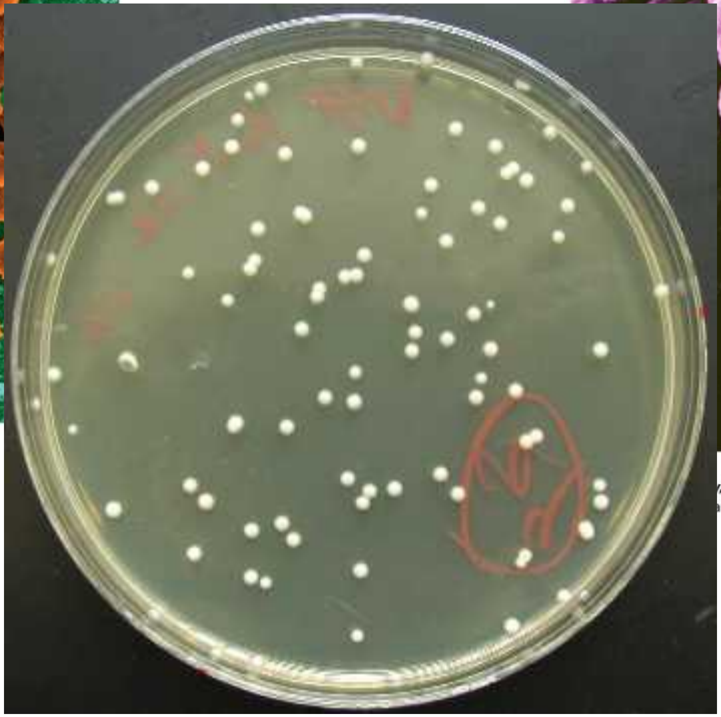
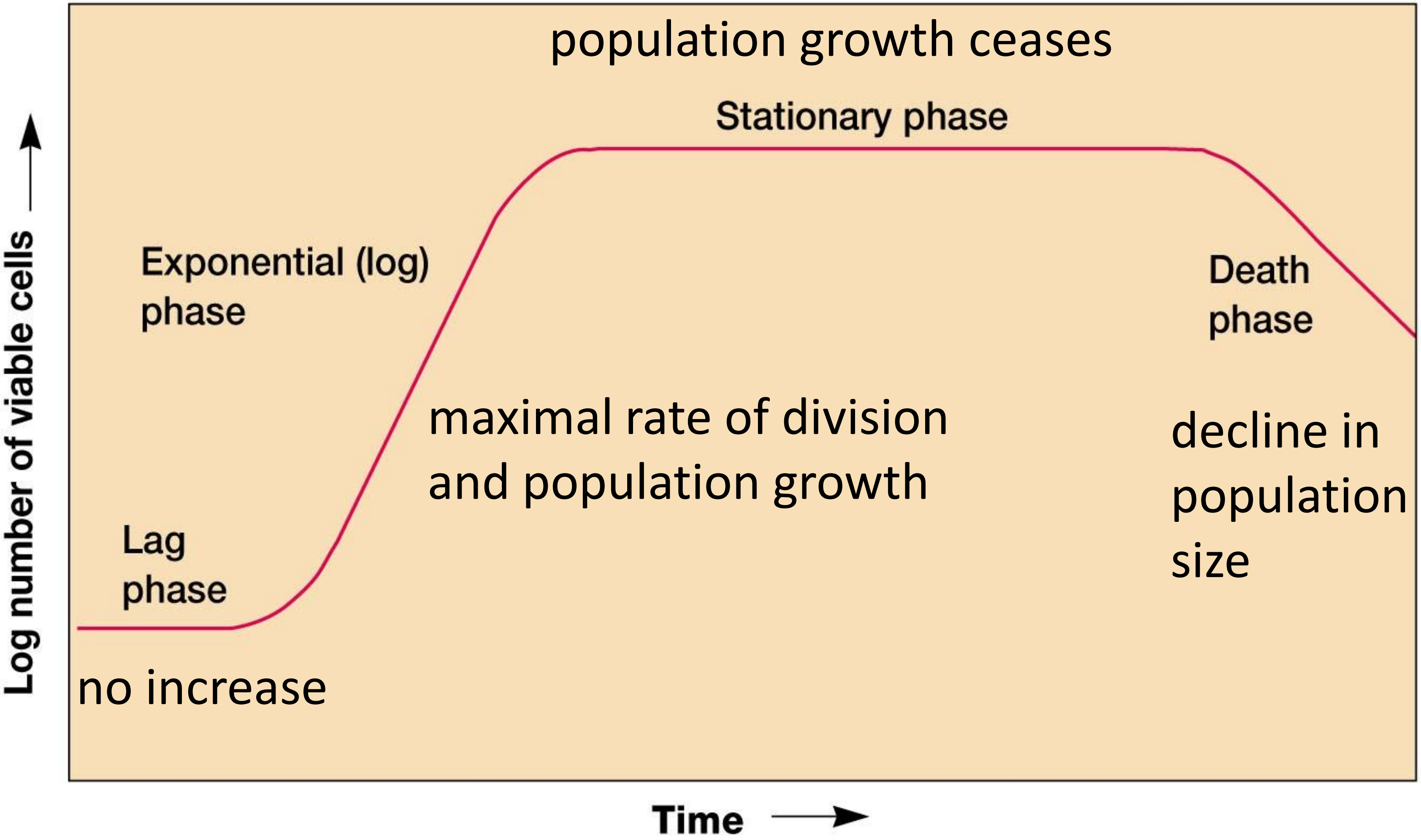


, Sixth Edition
nd Company



population growth ceases

maximal rate of division and population growth

decline in population size

no increase

The Mathematics of Growth

- Generation (doubling) time
  - time required for the population to double in size
- Mean growth rate constant
  - number of generations per unit time
  - usually expressed as generations per hour

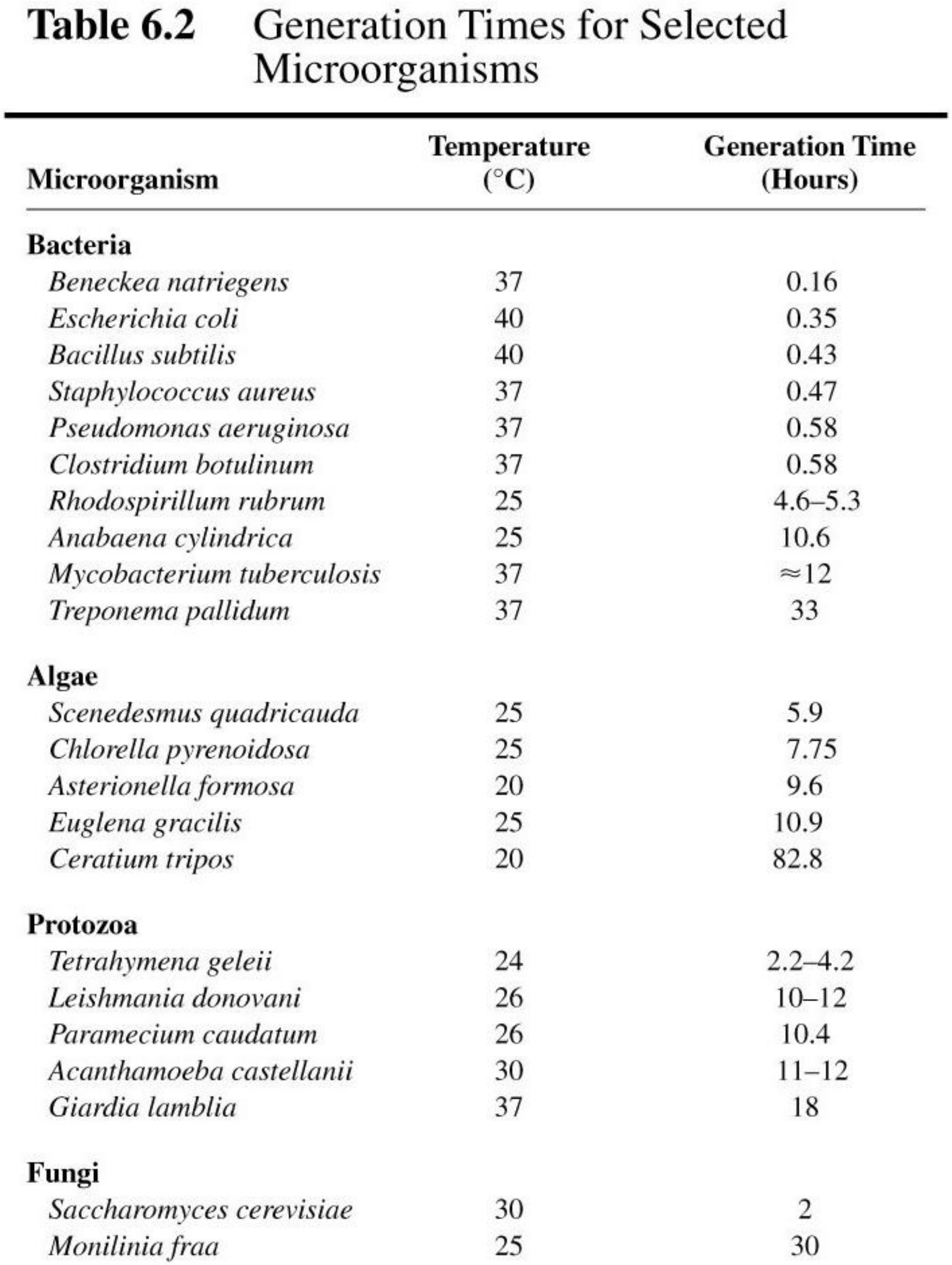
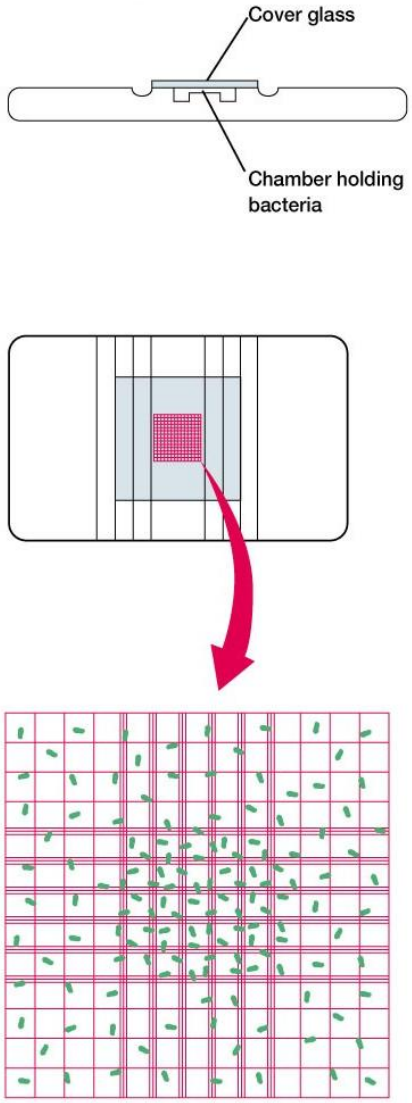


Table 6.2 Generation Times for Selected Microorganisms

Microorganism	Temperature (°C)	Generation Time (Hours)
Bacteria		
Beneckea natriegens	37	0.16
Escherichia coli	40	0.35
Bacillus subtilis	40	0.43
Staphylococcus aureus	37	0.47
Pseudomonas aeruginosa	37	0.58
Clostridium botulinum	37	0.58
Rhodospirillum rubrum	25	4.6–5.3
Anabaena cylindrica	25	10.6
Mycobacterium tuberculosis	37	≈12
Treponema pallidum	37	33
Algae		
Scenedesmus quadricauda	25	5.9
Chlorella pyrenoidosa	25	7.75
Asterionella formosa	20	9.6
Euglena gracilis	25	10.9
Ceratium tripos	20	82.8
Protozoa		
Tetrahymena geleii	24	2.2–4.2
Leishmania donovani	26	10–12
Paramecium caudatum	26	10.4
Acanthamoeba castellanii	30	11–12
Giardia lamblia	37	18
Fungi		
Saccharomyces cerevisiae	30	2
Monilinia fraa	25	30

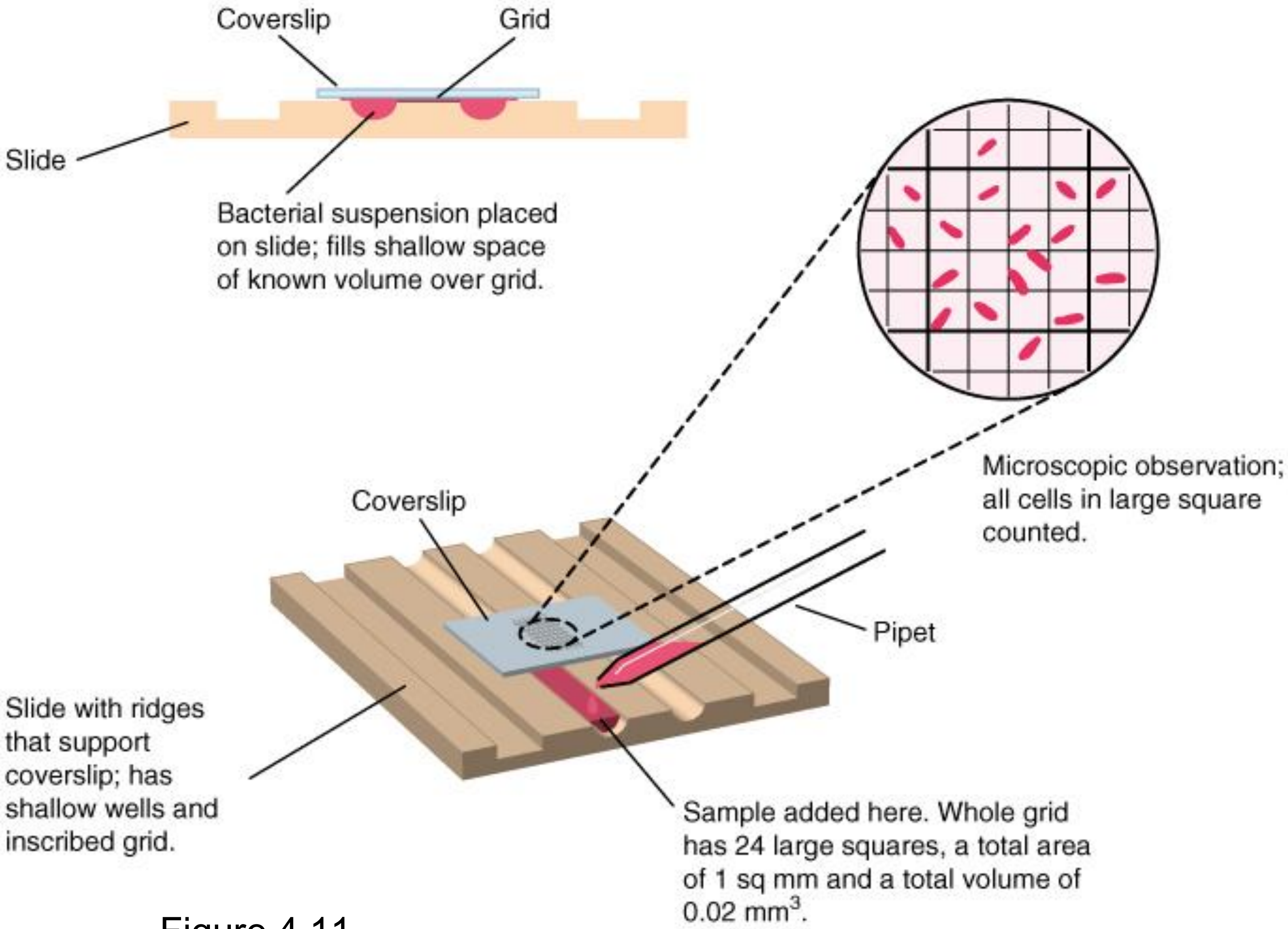
Measurement of Cell Numbers

- Direct cell counts
  - counting chambers
  - electronic counters
  - on membrane filters
- Viable cell counts
  - plating methods
  - membrane filtration methods



Counting chambers

Direct cell counts



Bacterial suspension placed on slide; fills shallow space of known volume over grid.

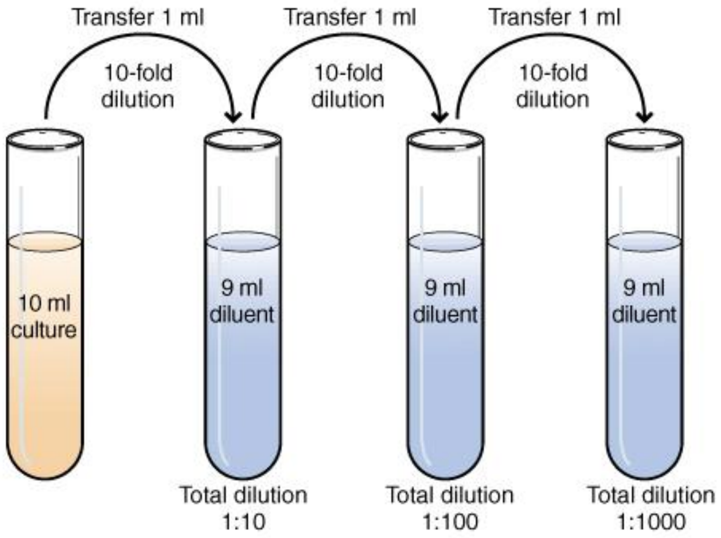
Microscopic observation; all cells in large square counted.

Slide with ridges that support coverslip; has shallow wells and inscribed grid.

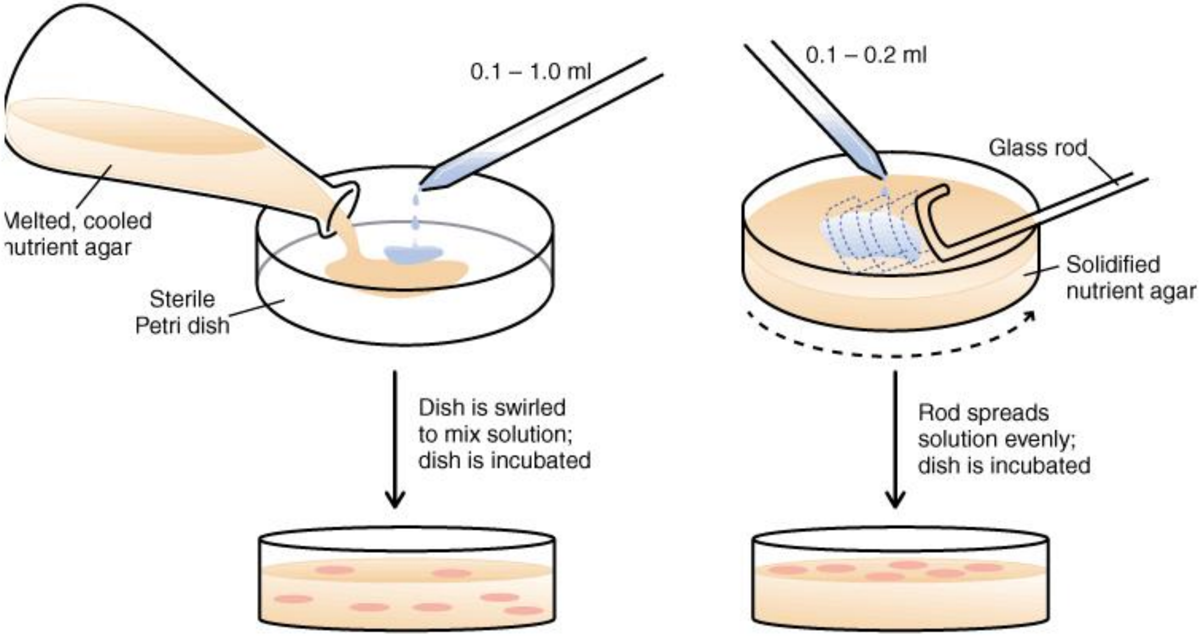
Sample added here. Whole grid has 24 large squares, a total area of 1 sq mm and a total volume of 0.02 mm^3.

Figure 4.11

Viable cell counts



(a) Serial Dilutions


0.1 – 1.0 ml
Melted, cooled nutrient agar
Sterile Petri dish
Dish is swirled to mix solution; dish is incubated
0.1 – 0.2 ml
Glass rod
Solidified nutrient agar
Rod spreads solution evenly; dish is incubated

(b) Pour Plate

(c) Spread Plate

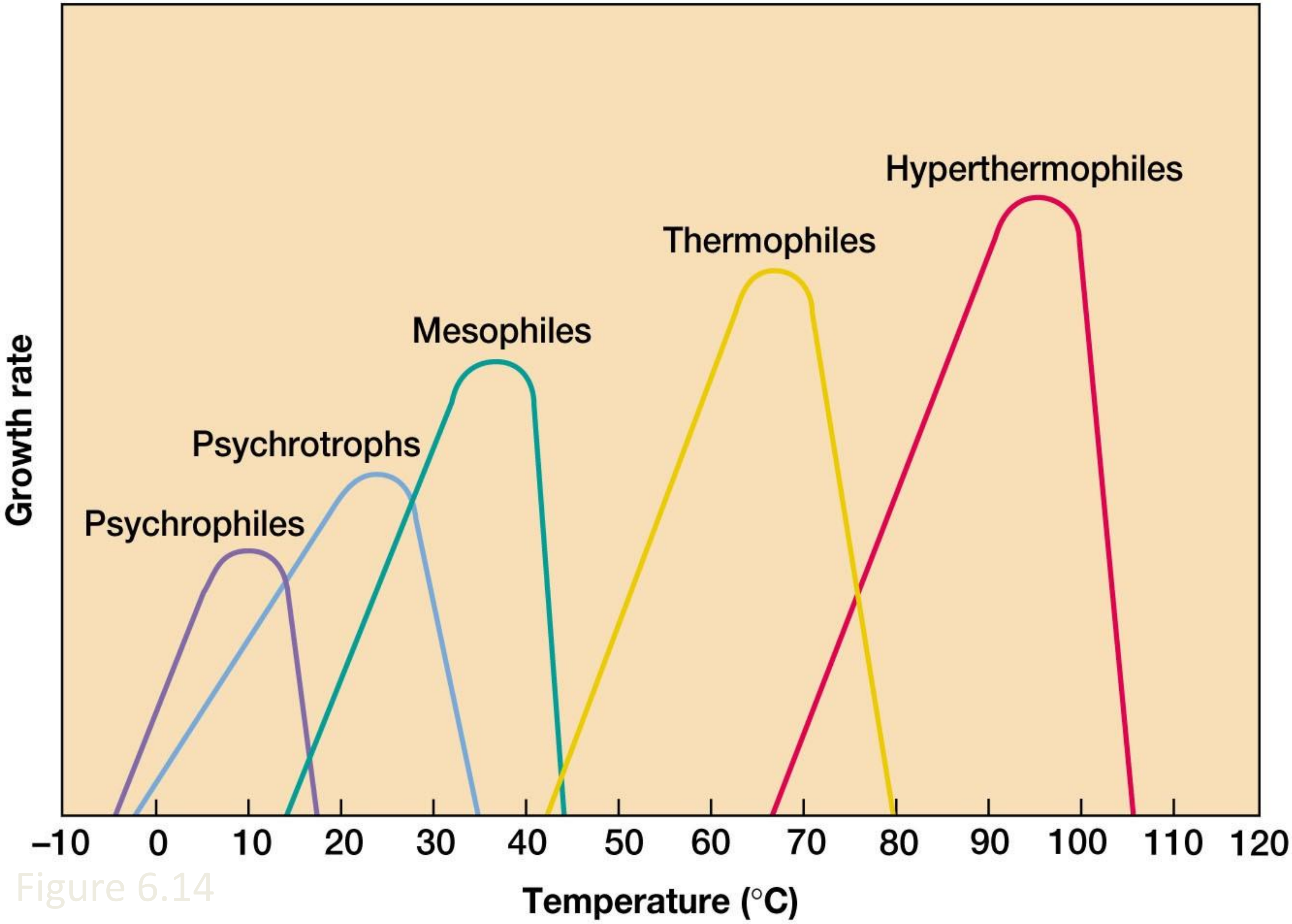
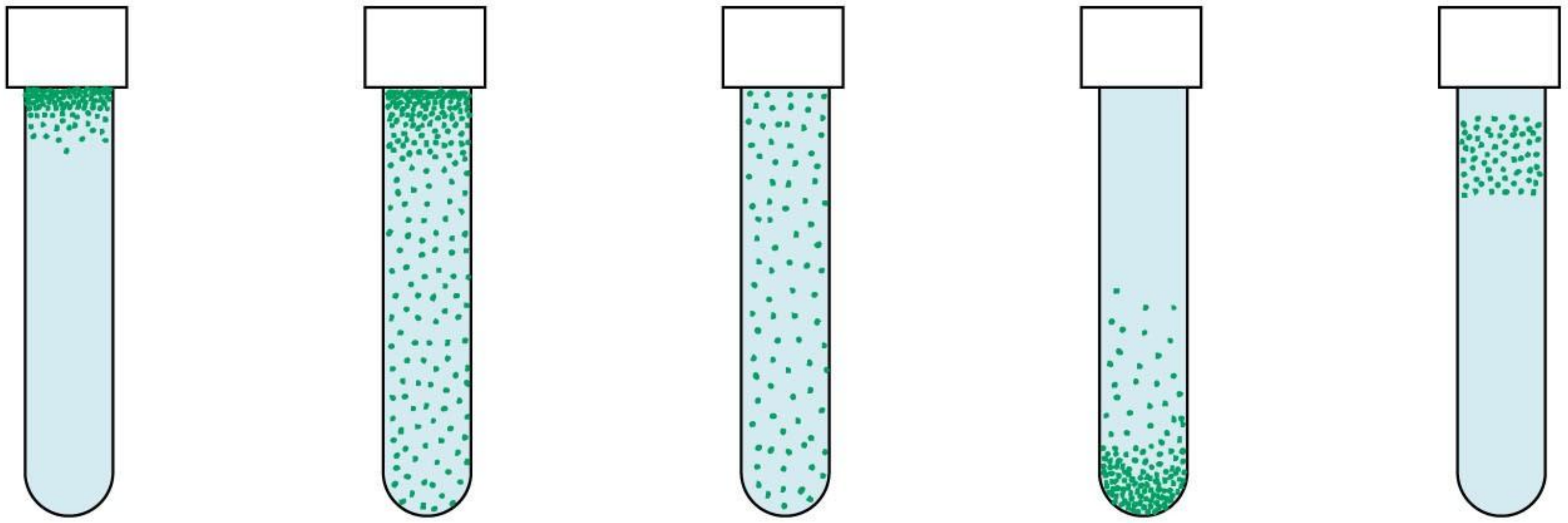

Hyperthermophiles
Thermophiles
Mesophiles
Psychrotrophs
Psychrophiles
Growth rate
–10
0
10
20
30
40
50
60
70
80
90
100
110
120
Temperature (°C)

Figure 6.14

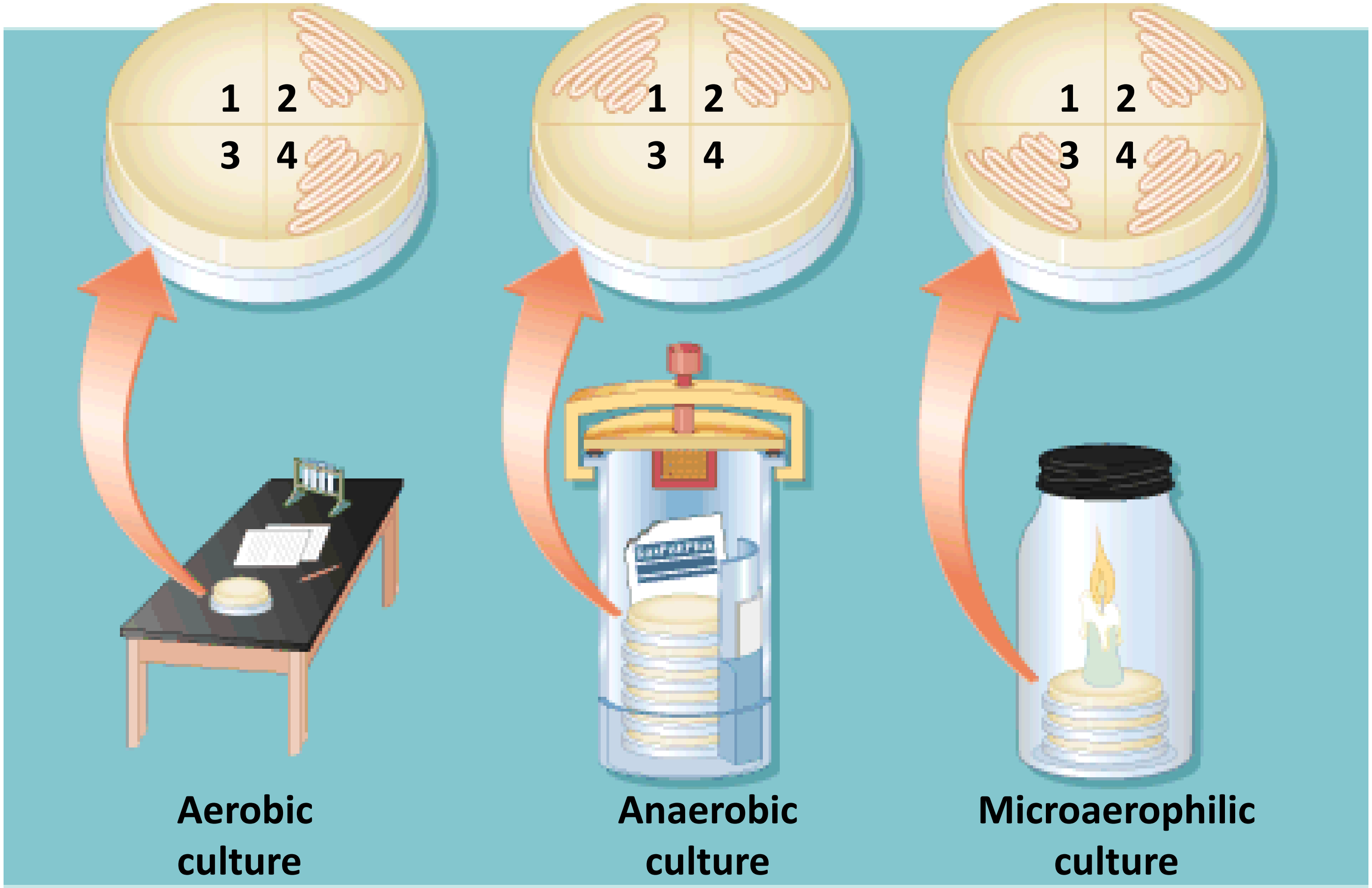


need oxygen	prefer oxygen	ignore oxygen	oxygen is toxic	< 2 – 10% oxygen
Obligate aerobe	Facultative anaerobe	Aerotolerant anaerobe	Strict anaerobe	Microaerophile

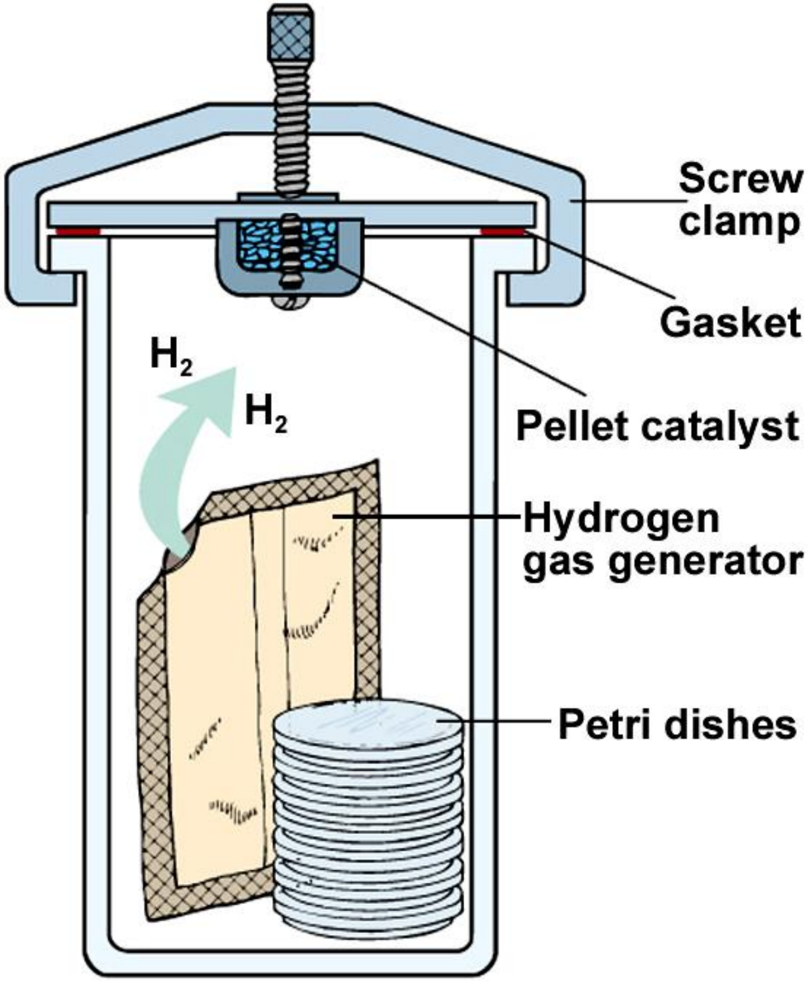
Enzyme content

+ SOD + Catalase	+ SOD + Catalase	+ SOD – Catalase	– SOD – Catalase	+ SOD +/– Catalase (low levels)

Oxygen requirements of bacterial isolate plate method



Anaerobes


Screw clamp
Gasket
Pellet catalyst
Hydrogen gas generator
Petri dishes
H_2
H_2

pH

- Acidophiles- optimum below pH 5.5
- Neutrophiles- optimum at pH 6-8
- Alkalophiles- optimum above pH 8

Synthetic or Defined Media

- all components and their concentrations are known

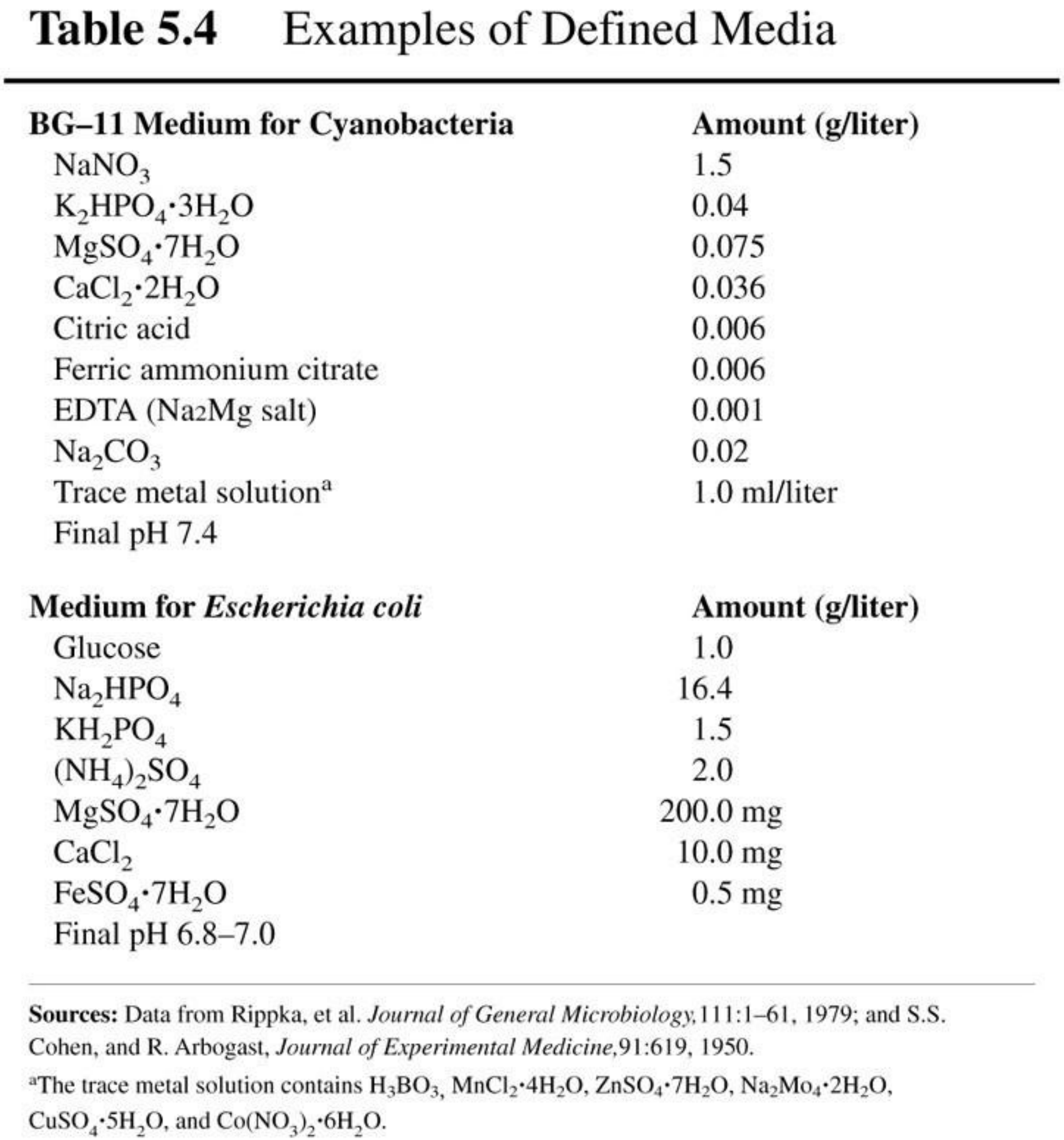


Table 5.4 Examples of Defined Media

BG–11 Medium for Cyanobacteria	**Amount (g/liter)**
$NaNO_3$	1.5
$K_2HPO_4 \cdot 3H_2O$	0.04
$MgSO_4 \cdot 7H_2O$	0.075
$CaCl_2 \cdot 2H_2O$	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA (Na_2Mg salt)	0.001
Na_2CO_3	0.02
Trace metal solution[a]	1.0 ml/liter
Final pH 7.4	

Medium for *Escherichia coli*	**Amount (g/liter)**
Glucose	1.0
Na_2HPO_4	16.4
KH_2PO_4	1.5
$(NH_4)_2SO_4$	2.0
$MgSO_4 \cdot 7H_2O$	200.0 mg
$CaCl_2$	10.0 mg
$FeSO_4 \cdot 7H_2O$	0.5 mg
Final pH 6.8–7.0	

Sources: Data from Rippka, et al. *Journal of General Microbiology,* 111:1–61, 1979; and S.S. Cohen, and R. Arbogast, *Journal of Experimental Medicine,* 91:619, 1950.

[a] The trace metal solution contains H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $Na_2Mo_4 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, and $Co(NO_3)_2 \cdot 6H_2O$.

Complex Media

- contain some ingredients of unknown composition and/or concentration

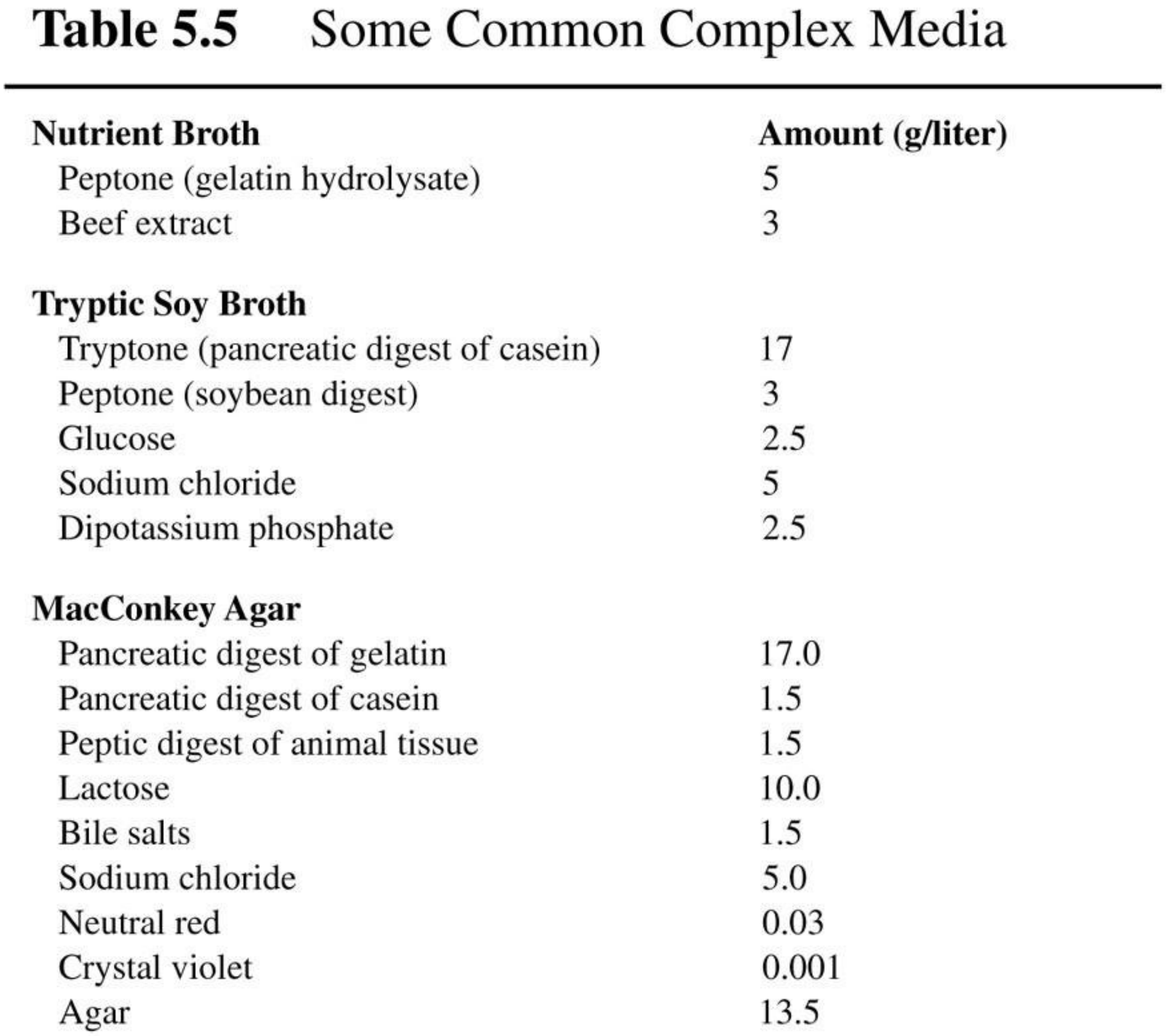


Table 5.5 Some Common Complex Media

Nutrient Broth	**Amount (g/liter)**
Peptone (gelatin hydrolysate)	5
Beef extract	3
Tryptic Soy Broth	
Tryptone (pancreatic digest of casein)	17
Peptone (soybean digest)	3
Glucose	2.5
Sodium chloride	5
Dipotassium phosphate	2.5
MacConkey Agar	
Pancreatic digest of gelatin	17.0
Pancreatic digest of casein	1.5
Peptic digest of animal tissue	1.5
Lactose	10.0
Bile salts	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

Prototrophs vs. Auxotrophs

- Prototroph
  - A species or genetic strain of microbe capable of growing on a minimal medium consisting a simple carbohydrate or CO_2 carbon source, with inorganic sources of all other nutrient requirements

- Auxotroph
  - A species or genetic strain requiring one or more complex organic nutrients (such as amino acids, nucleotide bases, or enzymatic cofactors) for growth

growth factors: organic compounds required in small amounts

- not every growth factor is required by all cells

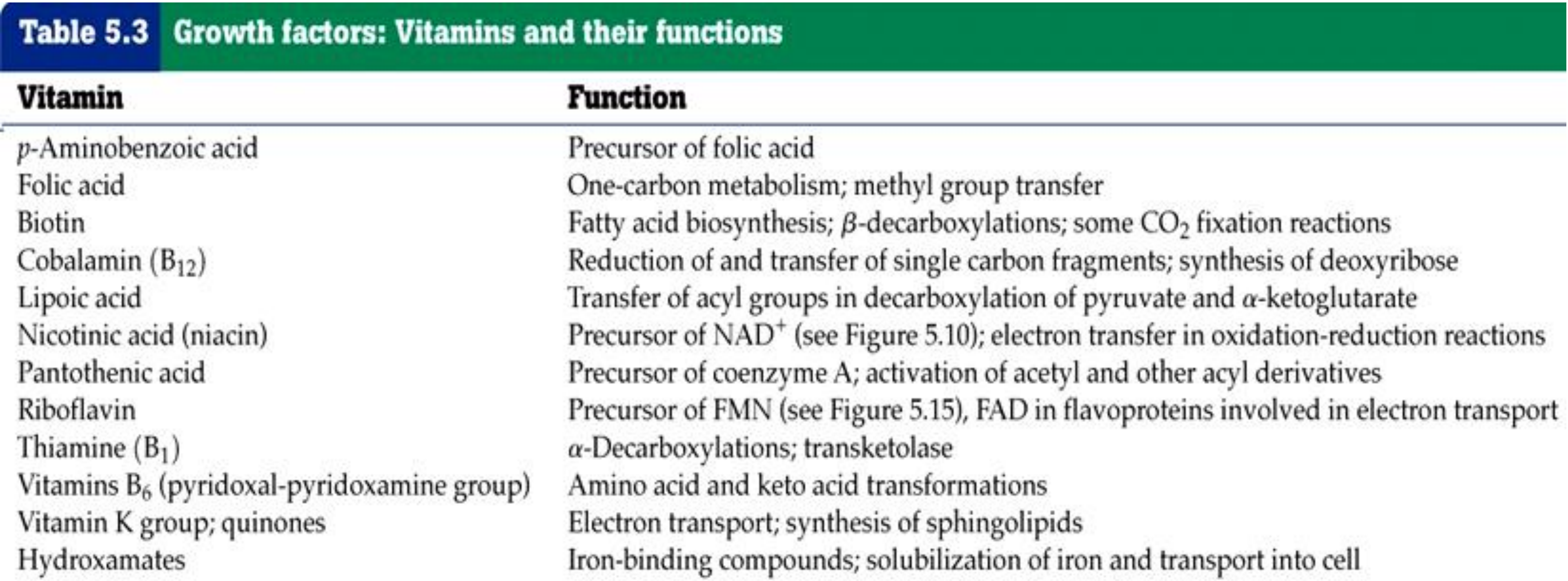
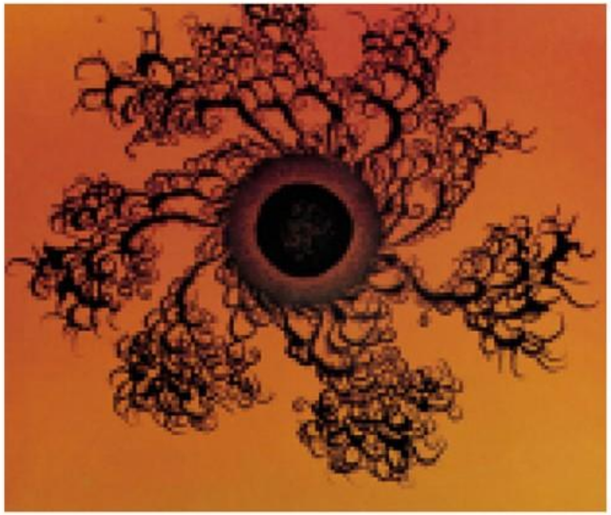


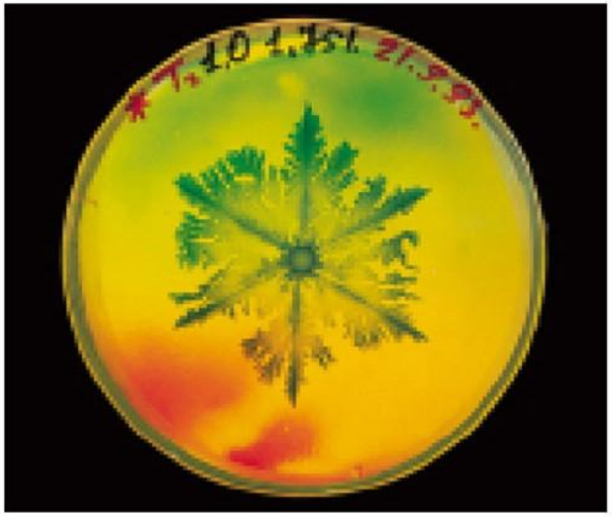
Table 5.3 **Growth factors: Vitamins and their functions**

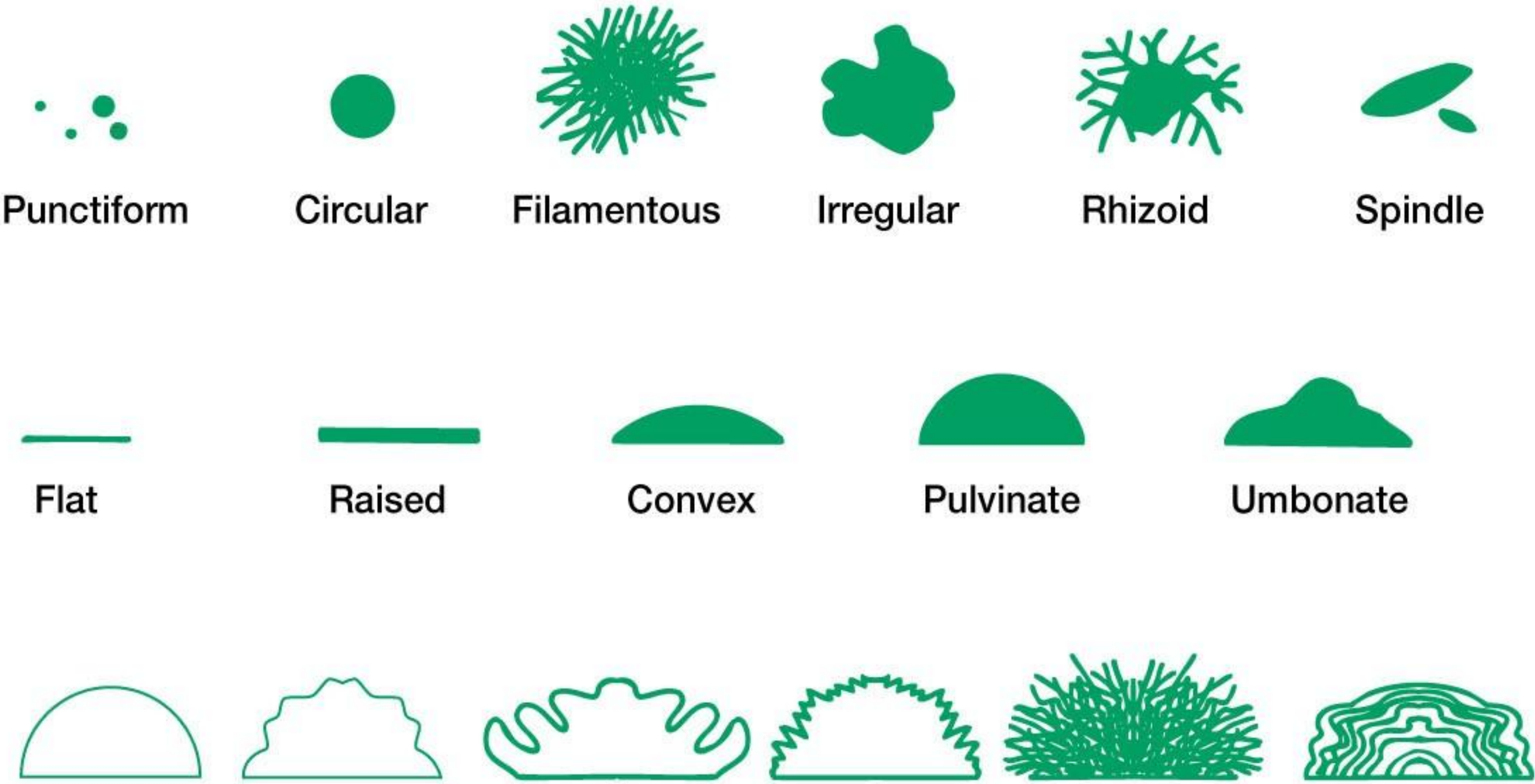
Vitamin	Function
p-Aminobenzoic acid	Precursor of folic acid
Folic acid	One-carbon metabolism; methyl group transfer
Biotin	Fatty acid biosynthesis; β-decarboxylations; some CO_2 fixation reactions
Cobalamin (B_{12})	Reduction of and transfer of single carbon fragments; synthesis of deoxyribose
Lipoic acid	Transfer of acyl groups in decarboxylation of pyruvate and α-ketoglutarate
Nicotinic acid (niacin)	Precursor of NAD^+ (see Figure 5.10); electron transfer in oxidation-reduction reactions
Pantothenic acid	Precursor of coenzyme A; activation of acetyl and other acyl derivatives
Riboflavin	Precursor of FMN (see Figure 5.15), FAD in flavoproteins involved in electron transport
Thiamine (B_1)	α-Decarboxylations; transketolase
Vitamins B_6 (pyridoxal-pyridoxamine group)	Amino acid and keto acid transformations
Vitamin K group; quinones	Electron transport; synthesis of sphingolipids
Hydroxamates	Iron-binding compounds; solubilization of iron and transport into cell

Colony Morphology and Growth

- individual species form characteristic colonies







Form

Punctiform | Circular | Filamentous | Irregular | Rhizoid | Spindle

Elevation

Flat | Raised | Convex | Pulvinate | Umbonate

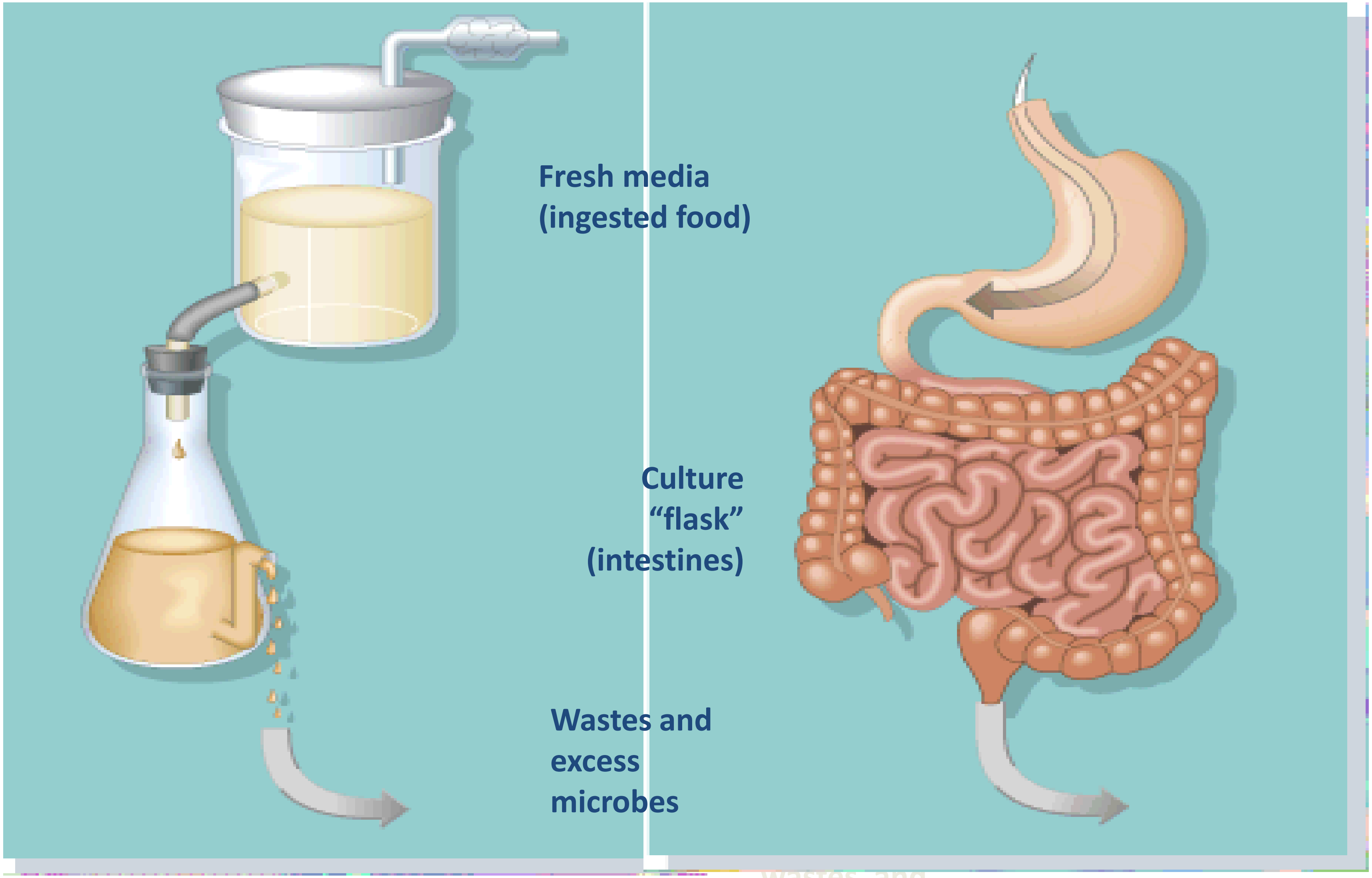
Margin

Entire | Undulate | Lobate | Erose | Filamentous | Curled

Two Methods for Culturing Microorganisms

- BATCH CULTURE
  - the microbe is grown in a closed vessel
  - typically in a test tube or flask
- CONTINUOUS CULTURE
  - the microbe is grown in vessel which has medium constantly added and spent medium constantly removed
  - performed in a **chemostat**

Continuous culture



Fresh media
(ingested food)

Culture
“flask”
(intestines)

Wastes and
excess
microbes

wastes, and
excess microbes

PROKARYOTE IDENTIFICATION

- Various techniques are employed to characterize and identify microorganisms
  - Phenotypic characteristics
    - Microscopic morphology
    - Metabolic differences
    - Serology
    - Fatty acid analysis
  - Genotypic characteristics
    - Nucleic acid probes
    - DNA amplification
    - rRNA sequencing

PHENOTYPIC CHARACTERISTICS

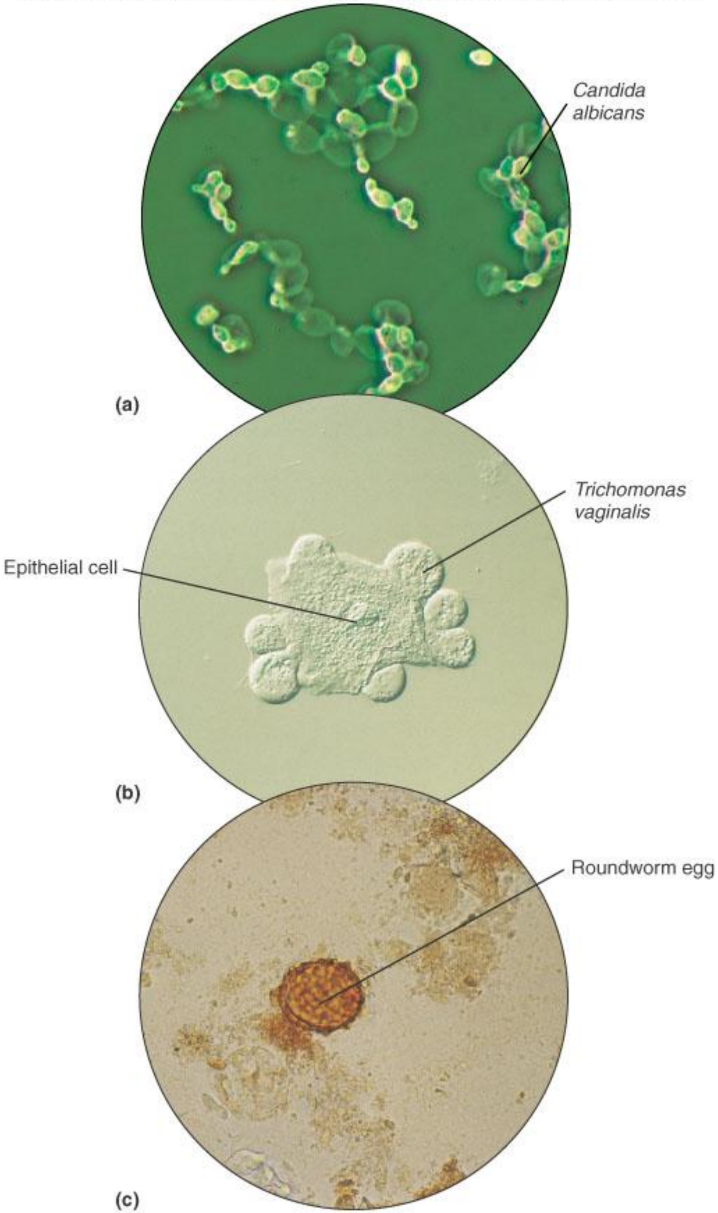
Microscopic morphology

- Size and shape
  - Readily determined by microscopic examination of a wet mount
  - Can determine whether the microbe is a prokaryote, fungus, or protozoan

PHENOTYPIC CHARACTERISTICS

Microscopic morphology

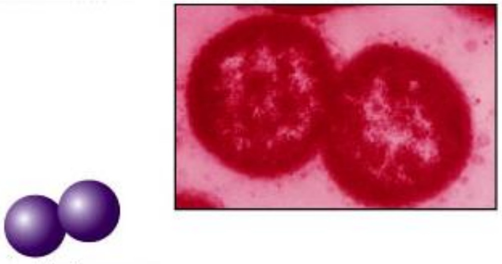
- Size and shape

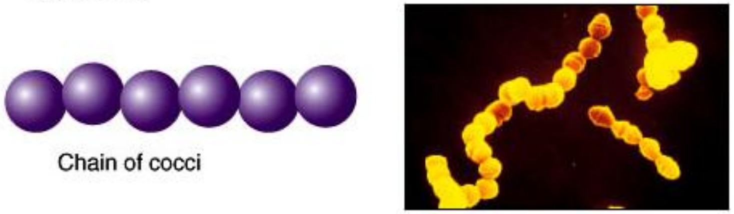


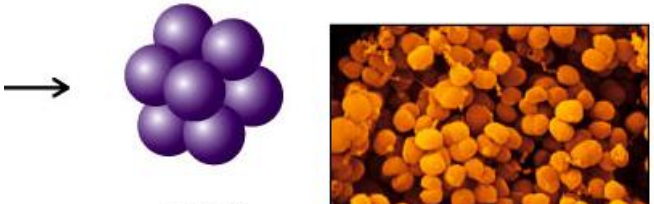
PHENOTYPIC CHARACTERISTICS

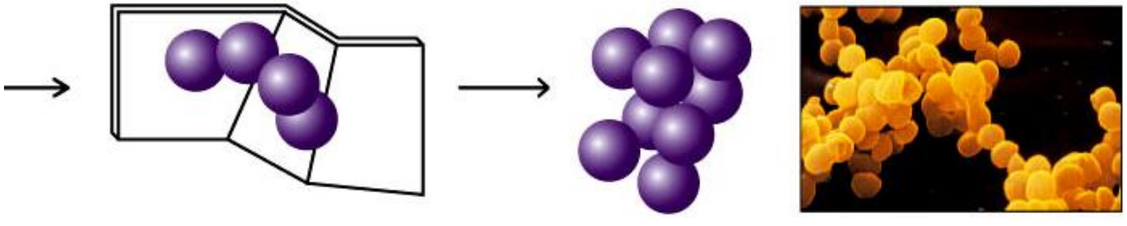
Microscopic morphology

- Cell groupings


Diplococcus


Chain of cocci

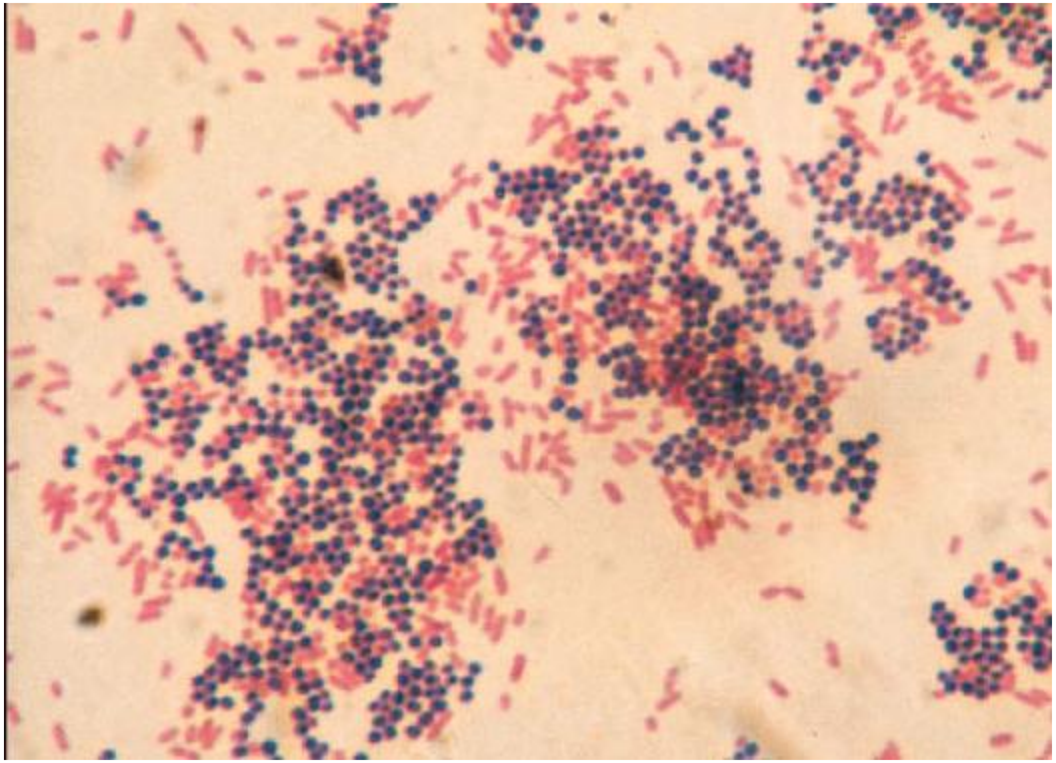

Packet


Cluster

PHENOTYPIC CHARACTERISTICS

Microscopic morphology

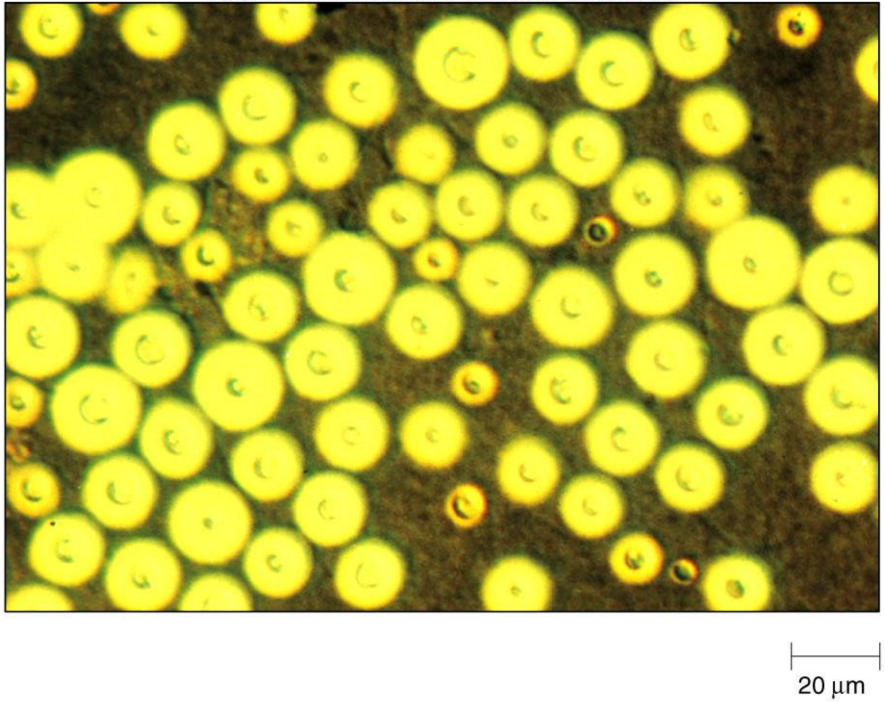
- Gram stain



PHENOTYPIC CHARACTERISTICS

Microscopic morphology

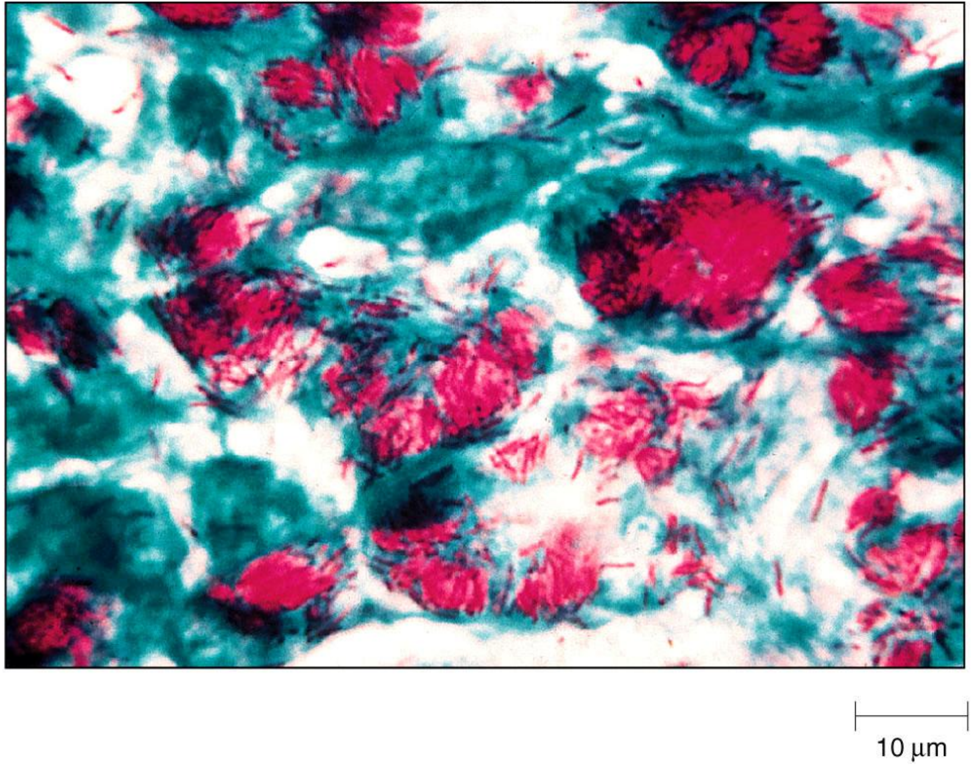
- Special stains
    - **Capsule stain**



PHENOTYPIC CHARACTERISTICS

Microscopic morphology

- Special stains
    - **Acid-fast stain** on sputum is diagnostic for tuberculosis



Results – CLSM of biofilm in CDFF

- Biofilms were heterogenous
- P.aeruginosa (green) dominates throughout the biofilm (e)
- S.aureus (blue) concentrates towards the surface of the biofilm (f)
- Other 2 species concentrate towards the middle and lower part of the biofilm

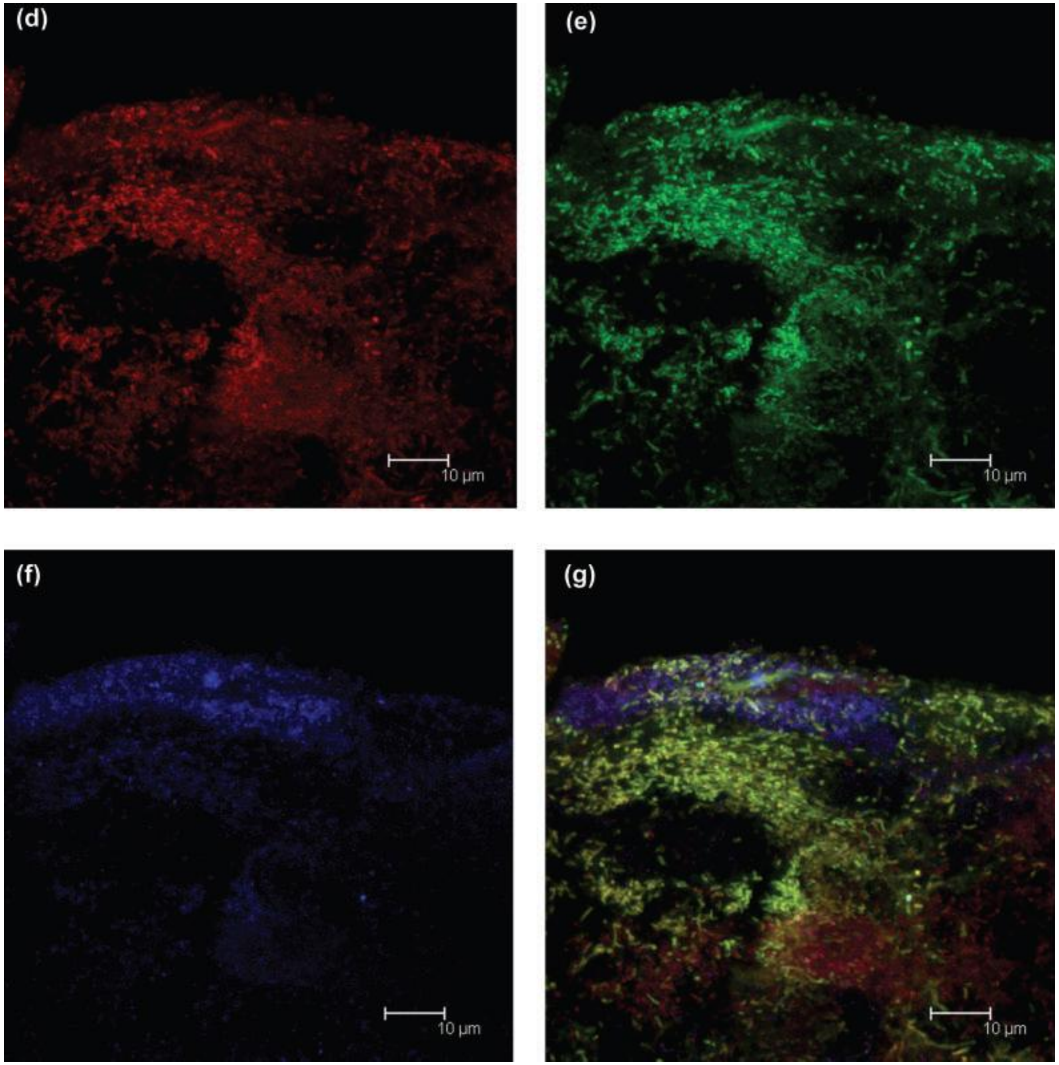
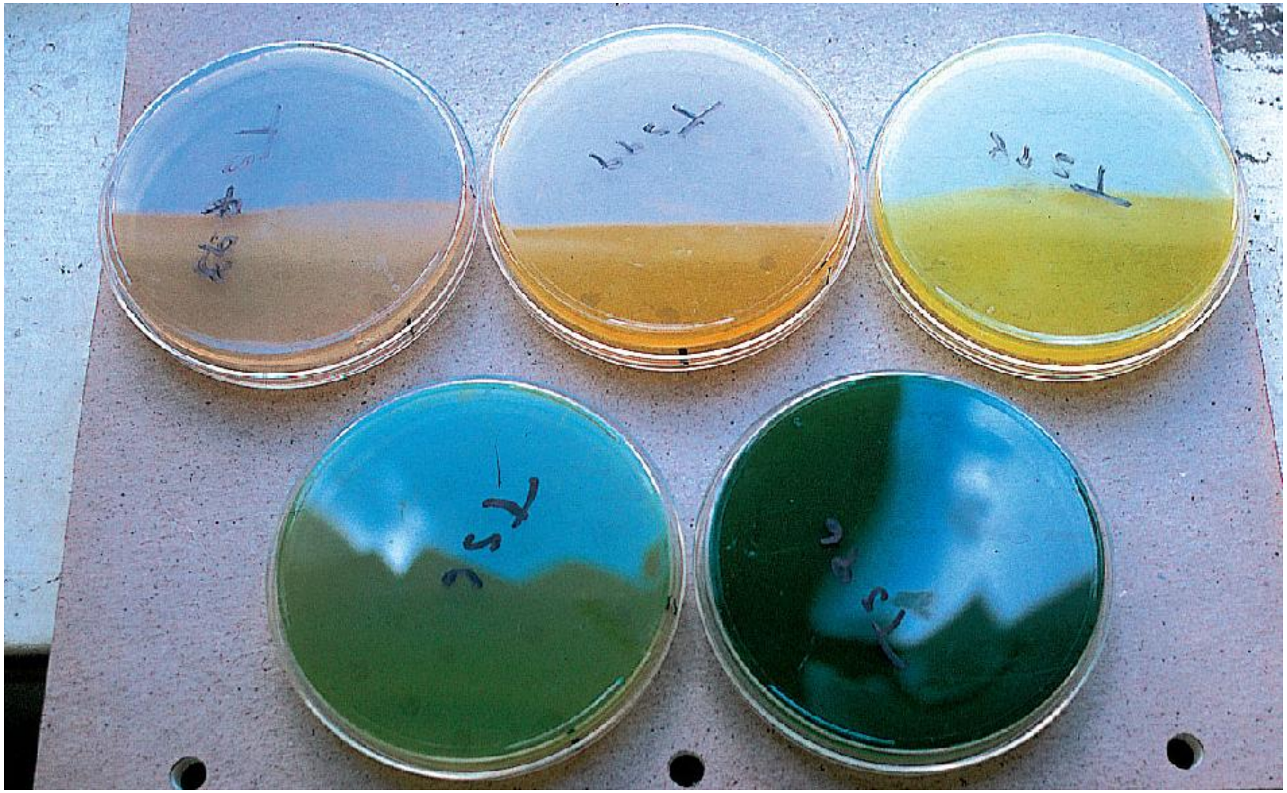


Figure 1: **d:** Bac-Unil probe with CY3 label shows all bacteria as red **e:** Psaer probe with FITC label shows *P.aeruginosa* green **f:** Sta probe with CY5 label show *S.aureus* blue **g:** multiplex PNA staining

PHENOTYPIC CHARACTERISTICS

Metabolic differences

- Colony morphology



PHENOTYPIC CHARACTERISTICS

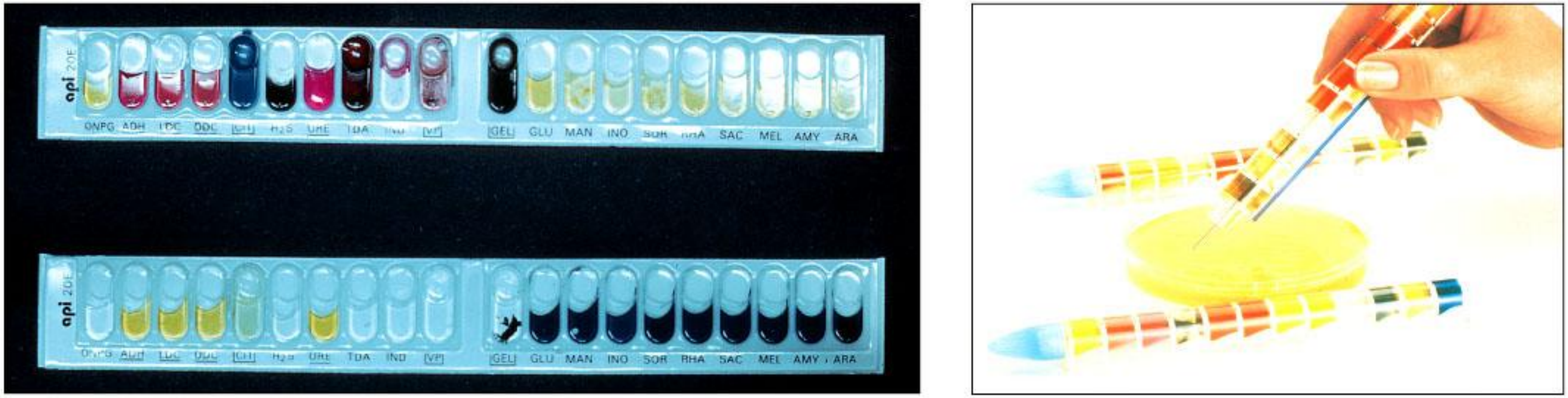
Metabolic differences

- Culture characteristics
  - Selective and differential media

PHENOTYPIC CHARACTERISTICS

Metabolic differences

- Biochemical tests
  - Generally necessary for more conclusive identification
  - Most rely on pH indicator or color change when a compound is degraded



(a)

(b)

PHENOTYPIC CHARACTERISTICS

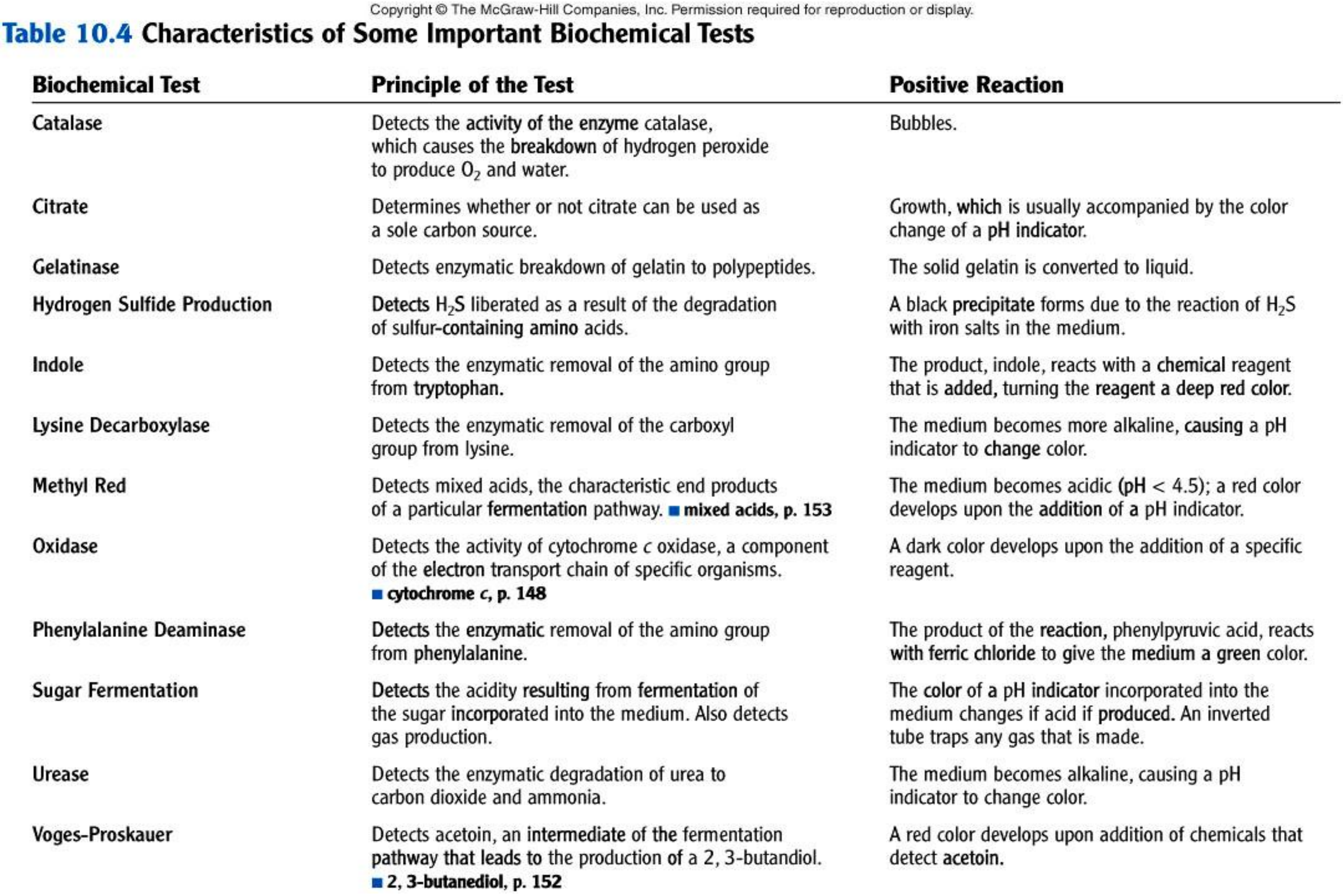
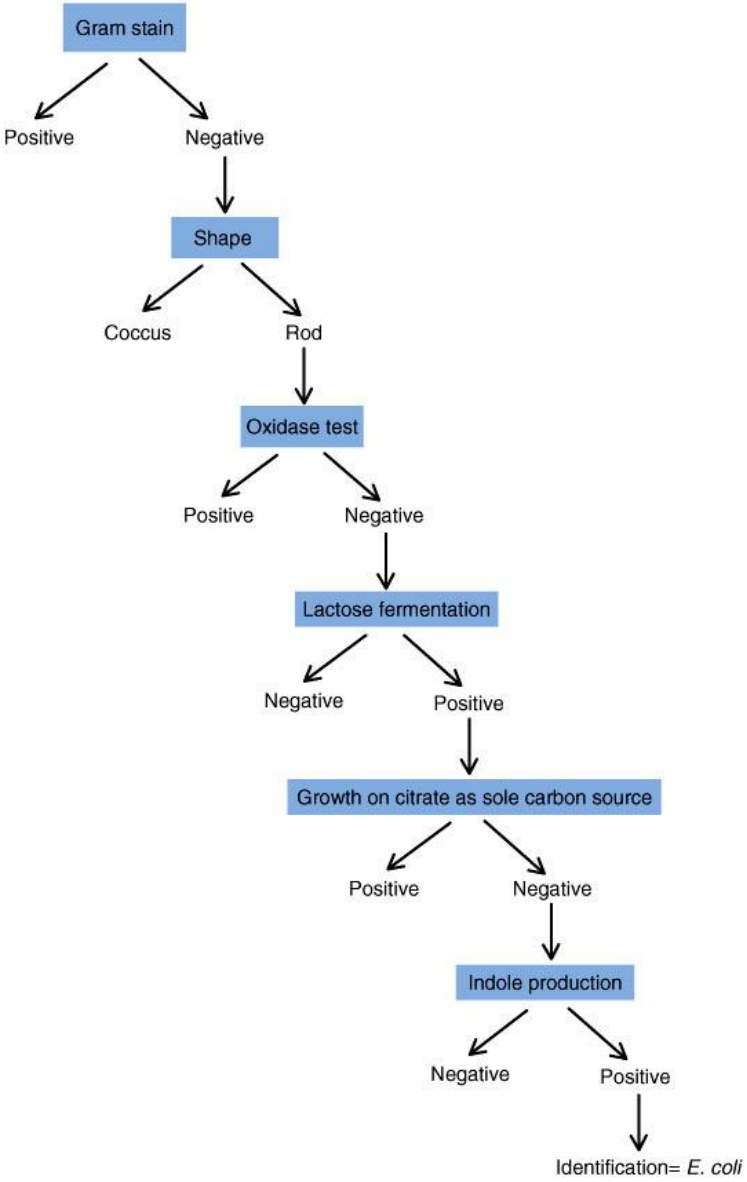


Table 10.4 Characteristics of Some Important Biochemical Tests

Biochemical Test	Principle of the Test	Positive Reaction
Catalase	Detects the activity of the enzyme catalase, which causes the breakdown of hydrogen peroxide to produce O_2 and water.	Bubbles.
Citrate	Determines whether or not citrate can be used as a sole carbon source.	Growth, which is usually accompanied by the color change of a pH indicator.
Gelatinase	Detects enzymatic breakdown of gelatin to polypeptides.	The solid gelatin is converted to liquid.
Hydrogen Sulfide Production	Detects H_2S liberated as a result of the degradation of sulfur-containing amino acids.	A black precipitate forms due to the reaction of H_2S with iron salts in the medium.
Indole	Detects the enzymatic removal of the amino group from tryptophan.	The product, indole, reacts with a chemical reagent that is added, turning the reagent a deep red color.
Lysine Decarboxylase	Detects the enzymatic removal of the carboxyl group from lysine.	The medium becomes more alkaline, causing a pH indicator to change color.
Methyl Red	Detects mixed acids, the characteristic end products of a particular fermentation pathway. ■ **mixed acids, p. 153**	The medium becomes acidic ($pH < 4.5$); a red color develops upon the addition of a pH indicator.
Oxidase	Detects the activity of cytochrome *c* oxidase, a component of the electron transport chain of specific organisms. ■ **cytochrome *c*, p. 148**	A dark color develops upon the addition of a specific reagent.
Phenylalanine Deaminase	Detects the enzymatic removal of the amino group from phenylalanine.	The product of the reaction, phenylpyruvic acid, reacts with ferric chloride to give the medium a green color.
Sugar Fermentation	Detects the acidity resulting from fermentation of the sugar incorporated into the medium. Also detects gas production.	The color of a pH indicator incorporated into the medium changes if acid if produced. An inverted tube traps any gas that is made.
Urease	Detects the enzymatic degradation of urea to carbon dioxide and ammonia.	The medium becomes alkaline, causing a pH indicator to change color.
Voges-Proskauer	Detects acetoin, an intermediate of the fermentation pathway that leads to the production of a 2, 3-butandiol. ■ **2, 3-butanediol, p. 152**	A red color develops upon addition of chemicals that detect acetoin.

PHENOTYPIC CHARACTERISTICS

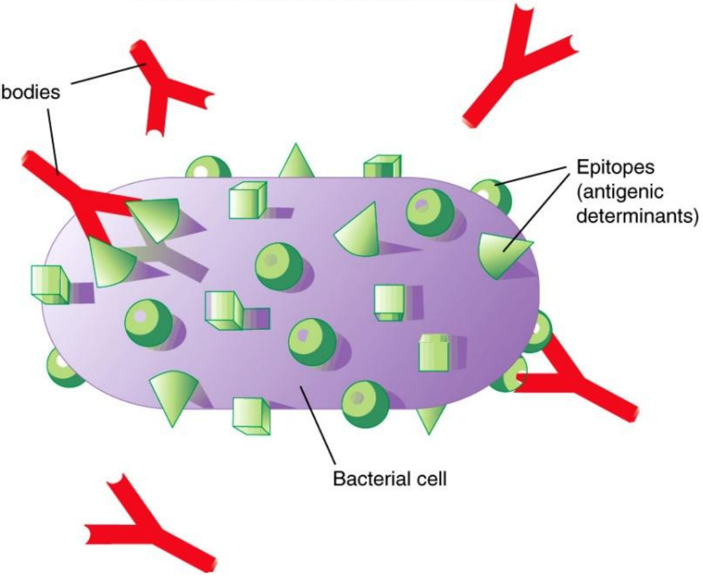
- Organisms are identified using a **dichotomous key**
  - Multiple biochemical and other tests are typically required
    - Multiple tests are generally run concurrently
      - Avoids waiting for incubation time for each test



PHENOTYPIC CHARACTERISTICS

Serology

- Proteins and polysaccharides of some bacteria can function as identifying markers
  - Generally molecules on surface
    - e.g., Cell wall, glycocalyx, fla
  - Detection is based upon the specific interaction between **antibodies** and these **antigens**
    - e.g., Rapid detection of *Streptococcus pyogenes*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PHENOTYPIC CHARACTERISTICS

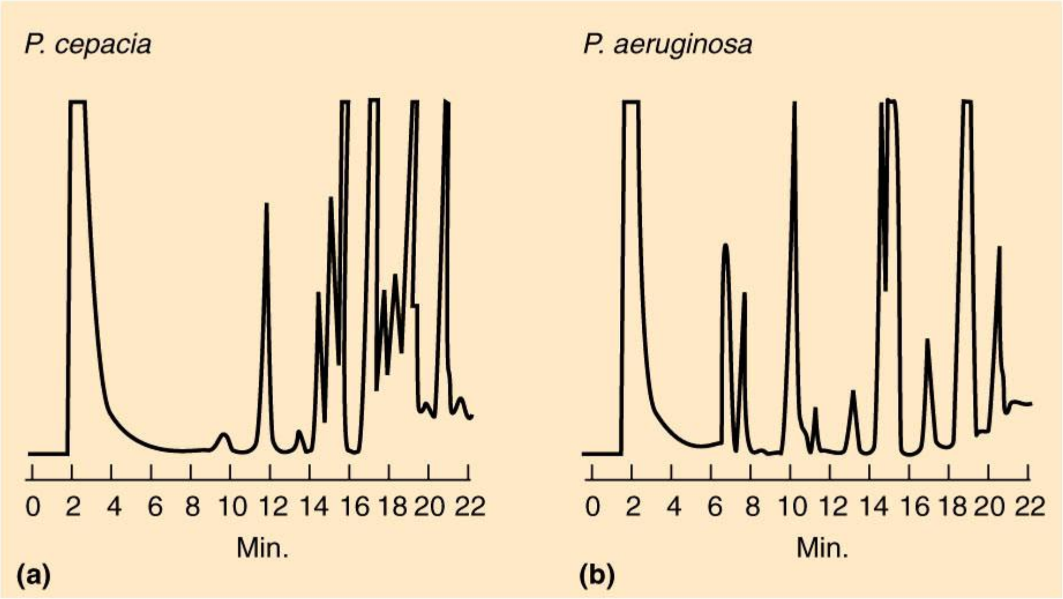
Fatty Acid Analysis

- Bacteria differ in the type and relative quantity of fatty acids that comprise their membranes
  - Can function as an identifying marker
    - Gram-negative bacteria possess fatty acids in both of their membranes
    - Gram-positive bacteria possess only a single membrane

PHENOTYPIC CHARACTERISTICS

Fatty Acid Analysis

- Cells are treated with NaOH and methanol
  - Fatty acids are released and converted into methyl esters
  - Methyl esters analyzed via gas chromatography
    - Profile compared to those of known species



GENOTYPIC CHARACTERISTICS

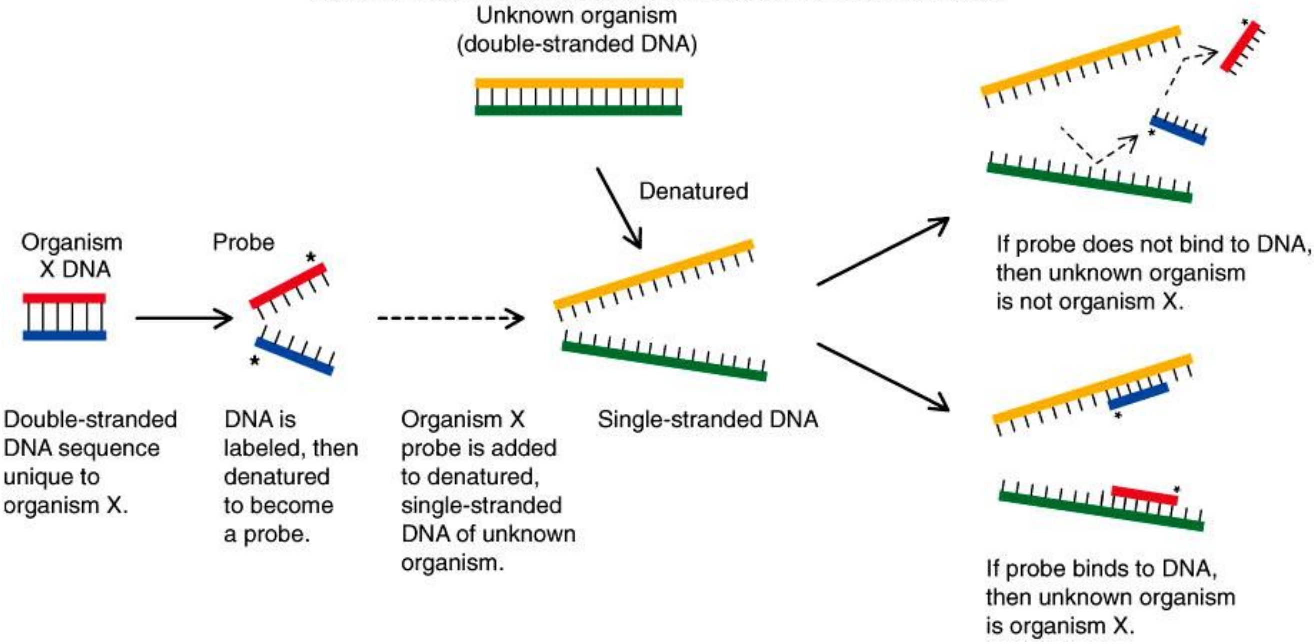
Nucleic acid probes

- Used to locate unique sequences
  - Single-stranded DNA (or RNA)
    - Generally labeled (radioactive or fluorescent)
  - Complementary to the sequence of interest
  - Observe and identify intact microorganisms
    - Fluorescence *in situ* hybridization (FISH)
  - Observe and identify samples
    - Generally preceded by DNA amplification

GENOTYPIC CHARACTERISTICS

Polymerase chain reaction

- Procedure
  - DNA is isolated, then denatured
  - Complementary primers are lengthened
  - DNA is doubled
  - Repeat ~30 times



Unknown organism (double-stranded DNA)

Denatured

Organism X DNA

Probe

Double-stranded DNA sequence unique to organism X.

DNA is labeled, then denatured to become a probe.

Organism X probe is added to denatured, single-stranded DNA of unknown organism.

Single-stranded DNA

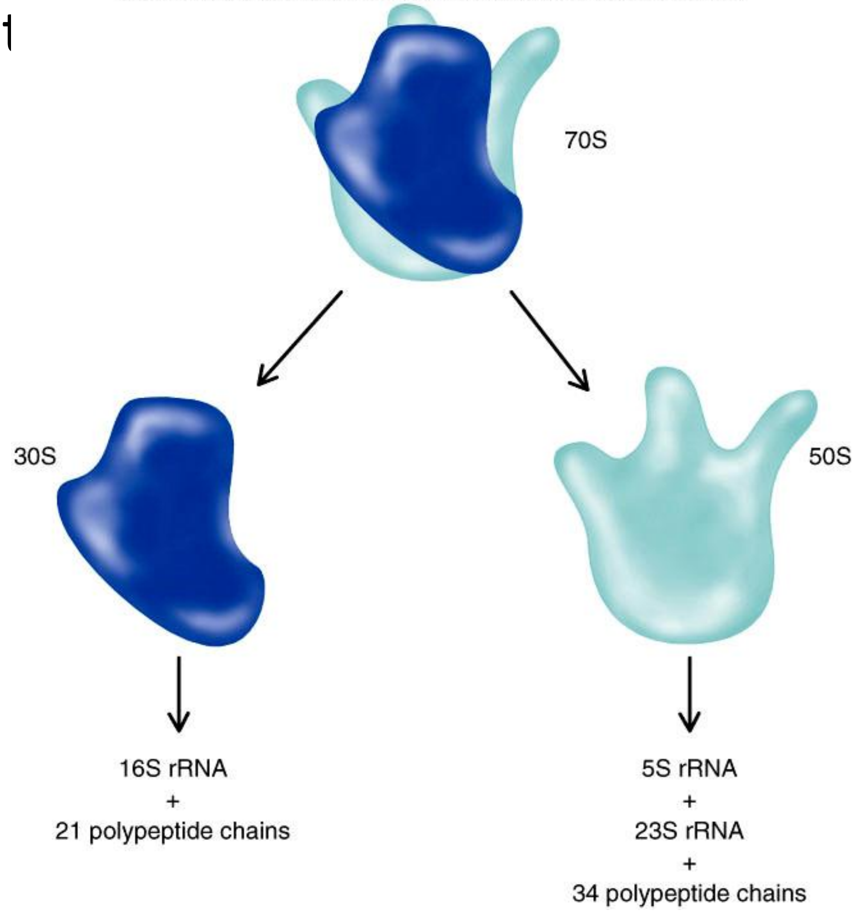
If probe does not bind to DNA, then unknown organism is not organism X.

If probe binds to DNA, then unknown organism is organism X.

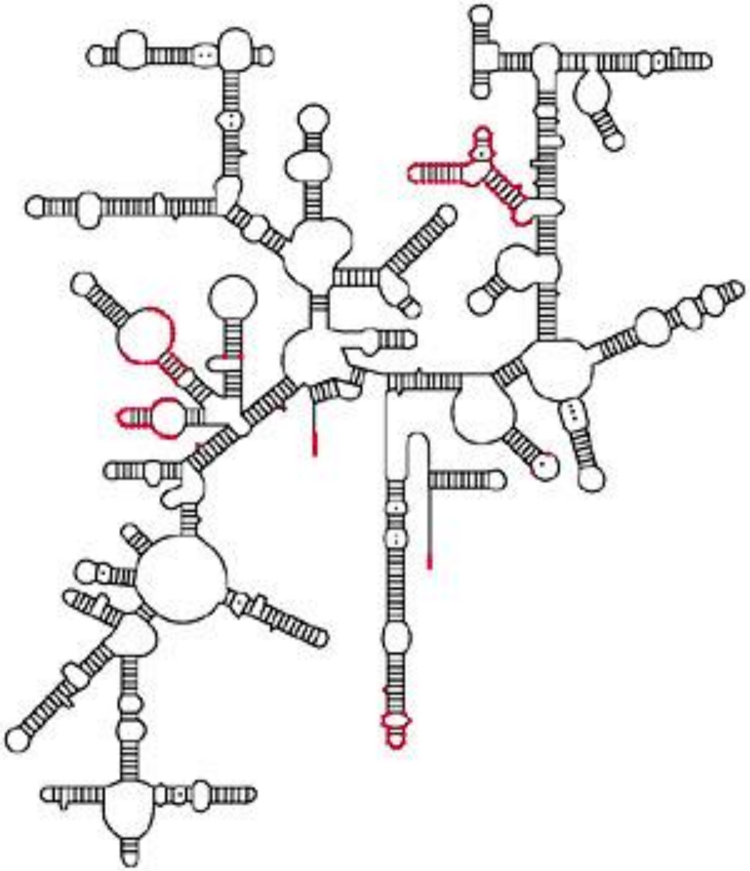
GENOTYPIC CHARACTERISTICS

Sequencing ribosomal RNA genes

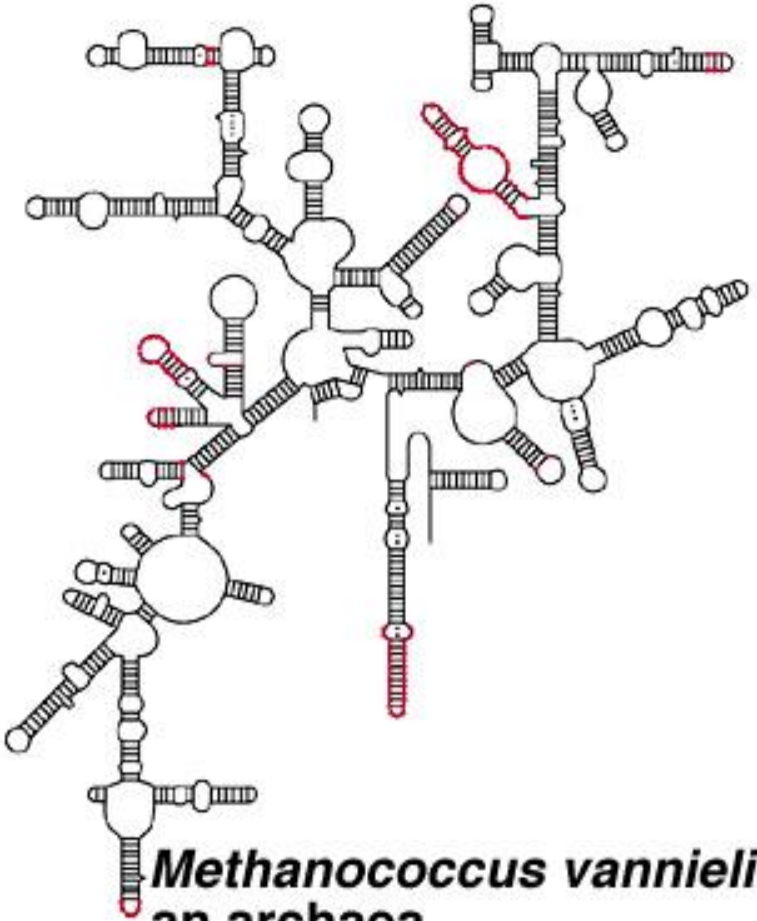
- Three rRNAs present in 70S bac
  - 5S, 16S, and 23S
- Evolutionarily highly conserved genes
  - Variable regions are used to identify an organism
- Particularly useful in identifying microbes that are difficult to culture



GENOTYPIC CHARACTERISTICS



Eschericia coli, a bacterium



Methanococcus vannielii, an archaea

🟥 Sites of variation in rRNA nitrogen base sequence